Comparative Study of Data Processing Techniques for Pancreatic Islets in Organ-on-Chip Applications

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Abstract: Organ-on-chip technology presents a promising platform to study complex physiological processes in a controlled environment. However organ-on-chip devices bring considerable constraints to online monitoring instrumentation. This study investigates methods for leveraging data from organ-on-chip systems designed for diabetic studies by processing recorded extracellular signals from pancreatic islets. The signal processing techniques used are designed to address the inherent constraints of microfluidics, particularly to provide online (real-time) readings and operate effectively in low Signal-to-Noise Ratio (SNR) conditions. This study assesses the performance of different algorithms using several detection approaches. Synthetic and experimental data were utilized to evaluate algorithm robustness to best account for biological variability. Among the algorithms tested, those based on frequency and time-frequency methods performed best when compared to conventional filtering and thresholding approaches, especially regarding robustness to noise and biological variability.

1 INTRODUCTION

Organ-on-Chips (OoC) are miniature systems replicating human organ structure and function on a microscale. Constructed using microfabrication techniques, these chips house animal or human cells that mimic organ function in micro- to milli-meter scale culture chambers. Microfluidic channels control the flow of nutrients, oxygen, and other substances, creating a dynamic microenvironment. Equipped with sensors for real-time monitoring, OoC technology is invaluable for studying physiological processes, modeling diseases, and testing drug responses in a more physiologically accurate *in vitro* setting (Clapp et al., 2021). This innovation holds the potential to advance drug development and personalized medicine by offering relevant and reliable experimentation models in a wide range of application cases (Mastrangeli et al., 2019). However, Organ-on-Chip technology faces notable challenges (Wikswo et al., 2013). First, integrating sensors onto the chip poses significant challenges due to its small size and the biological nature of the cultured material (Fuchs et al., 2021). Second, microfluidic equipment and flow introduce substantial noise during data acquisition. Additionally, the system requires fast data processing algorithms to address the demand for online monitoring (Moya et al., 2018) and feedback (either automated or performed by the experimenter).

This study was conducted in the context of OoC for diabetes and establishes a comparison of algorithmic solutions to process electrophysiological data from pancreatic islets recorded on MicroElectrode Arrays (MEA). In response to glucose, pancreatic beta cells activate and produce an oscillatory electrical signal that synchronizes with neighboring cells via a gap-junction protein (connexin36) (Lebreton et al., 2015). The resulting electrical signature resembles Action Potentials in shape, but with a broader and slower profile known as Slow Potentials (SP). SP oscillatory frequency (ranging between 0.2-2 Hz) corre-

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lates with the insulin production of Langherans islets and therefore gives precious insight of islet physiology (Jaffredo et al., 2021). This research aims at finding a precise, robust, and real-time method of measurement for the Slow Potential (SP) frequency, within the context of monitoring OoC devices for diabetes research.

2 MATERIAL AND METHODS

2.1 Electrophysiology Setup and Data Acquisition

The signal generated by beta cells was recorded using a Micro Electrode Array bonded to a PDMS microfluidic chip. Electrophysiological data were recorded using two widely used acquisition frontends : MCS 1060 preamplifier and USB-ME64 acquisition board as described in (Lebreton et al., 2015), or Intan RHD2132 pre-amplifiers (Abarkan et al., 2022) connected to a FPGA board for recording. All signal acquisition was performed at a sampling rate of 10 kHz. This study presents results from diverse (static and microfluidic) experiments and protocols.

2.2 Pancreatic Islet Signal Preliminary Analysis

Preliminary studies were carried out to determine the relevant parameters for SP identification. Fig. 1 illustrates key identification criteria for SP. In Fig. 1.A, the islet signal exhibits a pulsatile behavior, alternating between silent and active phases. SP are characterized by a specific high depolarization phase (Fig. 1.B, covered electrodes with SP) and occur in a frequency band of 0.2 Hz to 2.0 Hz (Fig. 1.C). With islet activity being modulated by glucose concentration, these measurements were conducted using a culture medium containing 11 mMol/l of glucose in which islets exhibit sustained activity. A Principal Component Analysis (PCA) and dbscan clustering was performed on metrics extracted from detected events : amplitude, frequency, amplitude of neighbouring events, frequency of neighbouring events, and waveform (waveform data points resampled to the dimensions of the shortest waveform detected) (Fig. 1.D). This is intended to highlight, based on event properties, clusters separating false detections of noisy events from physiological SP events. This clustering proved highly variable and did not - by itself - provide a robust method for separating biological signatures (SPs) from noise, which indicated that noise and SP share similar characteristics ; therefore, more sophisticated detection algorithms are needed to differentiate them.

2.3 Algorithmic Solutions

Taking into account signal characteristics, several algorithmic solutions were tested to evaluate the frequency of SPs. All signal processing and algorithmic tests were performed in Python 3.11.1 and libraries Scipy 1.10.0, Numpy 1.24.1, and PyWavelets 1.4.1.

The algorithmic solutions (described individually below) were tested and compared via parametric analyses mimicking a range of acquisition parameters (e.g. signal-to-noise ratio) and detection parameters (e.g. detection threshold). For a fair comparison of performance between algorithmic solutions, their detection parameters were normalized relative to their value of best performance (normalized parameter expressed as Δ/Δ_0 where Δ is the absolute value of the parameter and Δ_0 its value at best performance). Recorded signals were downsampled to 100 Hz to enhance computational efficiency without loss of relevant biological information considering the frequency range of interest for SP (0.2 Hz to 2.0Hz).

2.3.1 Offline Peak Detection

Slow Potentials were detected as local minima in the signal, found using Scipy's `find_peaks` (further referred to as FP) algorithm from Scipy 1.10.0 This function finds all local maxima by comparison of

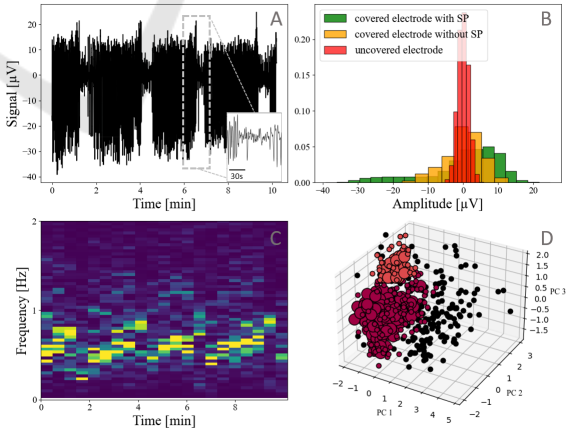

Figure 1: Characteristics of recorded islets electrophysiological data: (A) Filtered data from one MEA channel, (B) Amplitude histogram of three different channels (C) Spectrogram of A (D) Principal Component Analysis and DBSCAN clustering. True detections of SPs are shown in dark red, false detections caused by noise are shown in black and light red shows detections that could not be identified as eitheir noise or true detection.

neighboring values. As a baseline, prominence parameters were manually tuned to best match signal characteristics and maximize detection rate. Before peak detection, signals were filtered using Butterworth bandpass filter (0.2 Hz, 1st order to 2 Hz, 2nd order).

2.3.2 Online Peak Detection

To account for the real-time constraint of our study and provide a comparison reference with previous works, the next algorithms are either real-time or real-time-compatible. This algorithm (further referred to as RT), previously described in (Pirog et al., 2018), is also based on extremum detection with a prominence parameter. To include a validation period around every local extremum detected and avoid false detections caused by signal jittering, SP detected with this algorithms are processed some milliseconds after their actual appearance. Butterworth bandpass filtering was applied before detection (0.2 Hz, 1st order to 2 Hz, 2nd order).

2.3.3 Discrete Wavelet Transform Filtering

In this detection algorithm, signals were filtered using wavelet transformation before performing SP detection. The discrete wavelet transform (further referred to as DWT) (Unser and Aldroubi, 1996) is a mathematical transformation used to break down a signal into multiple sets of discretely sampled wavelets, that describe how the signal evolves over time within a specific frequency range. Therefore SP can be detected by selecting the coefficient in the frequency band of SP and applying prominence-based event detection. The DWT was applied to the input signal using Daubechies wavelets (db4) and 10 transform levels. Then for each set within the frequency band of interest (levels 0 to 6), a threshold was applied and only the coefficients above the threshold were kept. Finally, the filtered signal was constructed by applying an inverse wavelet transform, and peak detection was performed. The inputs parameters were the number of sets and the selected ones for filtering. Equally, the prominence parameter and a threshold value were required.

2.3.4 Generalized Likelihood Ratio Statistical Test

This algorithm (further referred to as GLR) aims at discerning between baseline noise and electrophysiological activity using a model-based approach. The Generalized Likelihood Ratio Test (GLR) as described in (Mansouri et al., 2018) was used to compare the goodness of fit of observed data against a statistical model of noise, thus helping detect changes in a signal and make decisions. The GLR method calculated a ratio of likelihoods between a target hypothesis (e.g., presence of a SP) and a reference hypothesis (e.g., baseline condition). This ratio served as a statistical test, indicating how well the data aligned with one hypothesis over another. Applying a threshold on this ratio signal yielded a SP detection index. This technique required a calibration phase (performed in the absence of electrophysiological signals, ie. inhibitory conditions for the islets), a detection threshold parameter, and an observation window to perform the GLR. Before processing, signals were filtered using a Butterworth bandpass filter (0.2 Hz, 1st order to 2 Hz, 2nd order).

2.3.5 Online Frequency Analysis

This last approach (further referred to as FFT) does not focus on event detection but rather aims at directly assessing signal frequency. To that end, a Fast Fourier Transform (FFT) algorithm was used with a 30 s sliding window and a 1 s step. An adaptive threshold was set according to a fraction of signal energy over the observation window, and peaks in FFT coefficients were detected when they exceeded that threshold. The frequency returned by the algorithm was the weighted average of the detected peaks in each observation window. Before computing FFT signals were filtered using a Butterworth bandpass filter (0.2 Hz, 1st order to 2 Hz, 2nd order).

2.4 Benchmarking

2.4.1 Performance Evaluation Metrics

Two distinct metrics were employed to evaluate the algorithms performances.

- Root Mean Square error (RMS): RMS error testifies for the difference between a model and an observation. RMS error was calculated as the square root of the mean of the squares of the differences between predicted values and observed values.
- Maximum of cross correlation: Cross correlation is a measure of similarity of two series, as a function of the displacement of one relative to the other. Cross correlation was computed using Numpy 1.24.1 then the maximum value was taken.

2.4.2 Test Scenarios

Detection algorithms were benchmarked across several test scenarios, to account for the inherent sig-

nal processing difficulties encountered in electropysiological signals recorded in microphysiological systems. First, synthetic signals mimicking the properties of electrophysiological data were used as test inputs : as the frequency of the test signal was well defined for each instant, it could be compared to measured outputs. The test signal consisted in a sine modulated in frequency and amplitude within the physiological range of the signal (ie. modulated frequency from 0.2 to 1.2 Hz and amplitudes between 5 to 30 μV) and reproducing the alternating silent/active phases. Gaussian white noise was added (SNR $\sim$ 5 dB) to mimic measurement noise.

Parametric Study of Detection Parameters. The sensitivity and variability of measured outputs in response to changes in measurement parameters was evaluated in a parametric study of their detection parameters. First, a preliminary parameter space study was conducted to evaluate the settings leading to best detection performance. Then, the most influential parameter (event-based methods FP, RT, DWT, GLR : the detection threshold ; frequency-base method FFT : the adaptive threshold) was systematically varied in a defined range (0.1 to 10 times) around its point of best performance. This study aimed at highlighting the immunity, or lack thereof, of the benchmarked algorithms to their detection parameters in an effort to minimize biases caused by algorithm configuration.

Parametric Analyses on Signal-to-Noise Ratio. To study the behaviour of the tested algorithms as the signal-to-noise ratio (SNR) deteriorates, a parametric study was conducted with added white noise, for a SNR ranging from 45dB to -10dB. The white noise applied during this study replaces the Gaussian white noise originally set in the test scenario.

Validation Study. Finally, all algorithms were tested on electrophysiological recordings of pancreatic islets, to account for experimental and biological variability. To provide a reference for performance evaluation, recorded signals were manually annotated with SP events and converted to a reference signal describing the expected frequency measurements. Furthermore, to emulate increasingly poor recording conditions and monitor detection performance loss, a parametric study was again done with added white noise of increasing intensity (SNR from 40 to 0 dB).

3 RESULTS

3.1 Parametric Study on Synthetic Data

The robustness of each algorithm against noise and biological variability was evaluated by adjusting their most sensitive parameter: the detection threshold. Fig. 2.A highlights the detection performance when tuning this parameter. The prominence-based algorithms RT and FP yielded low error when the threshold parameter was properly tuned, but were highly sensitive to its variations. Indeed, false detections abruptly occured when small variations were applied to the detection threshold. FFT exhibited lower performance but demonstrated very strong resilience to variations in the threshold parameter, as performance indicators remained almost constant regardless of the threshold value. On the other hand, DWT and GLR consistently delivered low error rates when compared to the other algorithms, and maintained excellent performance across a broad range of threshold values. Fig 2.B reports the influence of noise on detection performance. Results are consistant with Fig 2.A as the FP and RT algorithms showed high sensitivity to noise contrarily to FFT that showed very little perturbation. GLR and DWT still performed better than FP and RT at low SNR but were less robust than FFT.

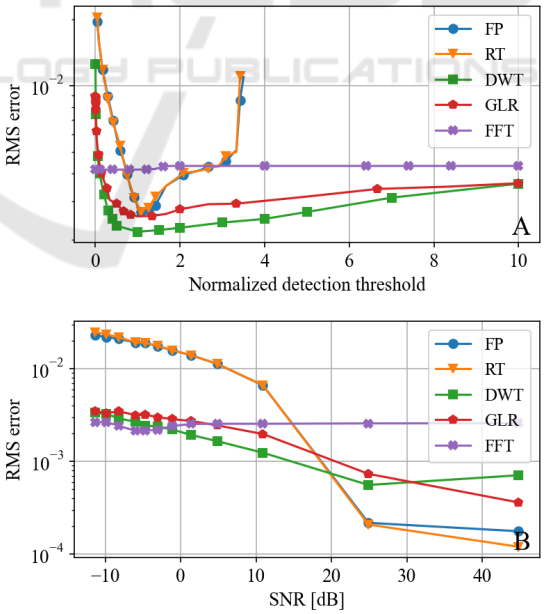

Figure 2: Evaluation of the algorithms (FO, RT, DWT, GLR, FFT) on synthetic signals. (A) Parametric analysis on the detection threshold (detection threshold was normalized according to Δ/Δ_0 where Δ_0 is the threshold at best performance and Δ the threshold). (B) Parametric analysis on the test signal SNR.

3.2 Parametric Study on Recorded Data

Next, the same technique was applied to recorded electrophysiological data. Recorded signals were manually annotated with SP events to serve as a reference for performance evaluation (Fig. 3).

As depicted in Fig. 3, the Fourier transform algorithm (FFT) appeared to be the most robust to noise. Both peak-finding algorithms (FP and RT) performances degraded quickly under higher noise conditions, despite maintaining a good overall correlation with the reference signal. The GLR algorithm performed the least favorably when compared to FFT and DWT. DWT showed good error results but exhibited a slightly lesser correlation fit compared to FFT.

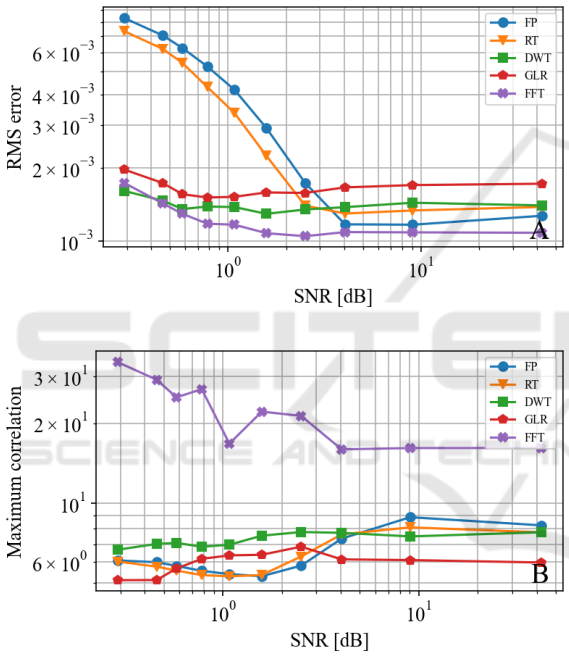

Figure 3: Evaluation of the algorithms (FO, RT, DWT, GLR, FFT) on recorded data with added white noise to change SNR. (A) RMS error. (B) Maximum of cross-correlation.

3.3 Validation Study

Finally the algorithms were tested on three recordings from different experiments and electrodes to account for the experimental and biological variability.

The overall behaviour of each algorithm matches the frequency variation of the signal but their performance level varies (Fig. 4). The detection follows the variations in glucose concentration ie the SP frequency increases from 4 to 12 mM and decreases from 12 to 4 mM. The lesser performance of GLR can be explained by a poor match between the noise

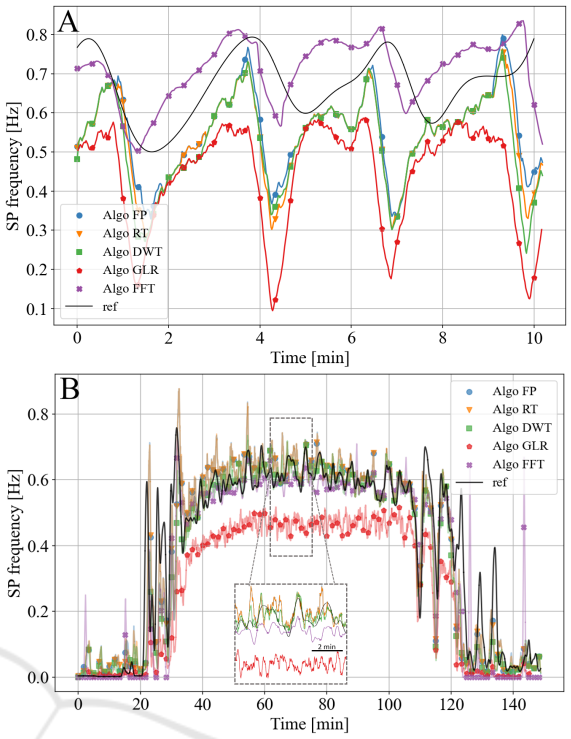

Figure 4: Comparison of the different algorithmic outputs to a manually constructed reference signal (both averaged over a 30 s window). (A) Single channel at constant glucose concentration (11 mM). (B) Average from two channels with variable glucose level (4 to 12 mM).

hypotheses made and the actual distribution recorded during the silent phases used for calibration.

4 DISCUSSION

This study offers insights into signal processing approaches for pancreatic islets in organs-on-chips. Results in Fig.5 show clear superiority of the FFT algorithm exhibiting lower RMS error and higher correlation with the reference. Performance with GLR was comparable with other algorithms, provided the noise model adequately matched the noise distribution of the experiments, and otherwise declining sharply. FP and RT showed good performances if correctly tuned but were highly dependent of SNR shown in Fig. 2 and 3. In comparison DWT filtering showed similar performances but higher immunity to noise. Nevertheless, the evaluation process on recorded data is limited by the quality of reference signals. Considering the complexity and variability of organ-on-chip devices, generating a reliable gold reference is a significant challenge. Fig.5 was obtained using three reference signals, where SP were hand marked, a common procedure in electrophysiology. Therefore the

so-called reference can only be used as an indicator subject to human error and not as an absolute reference.

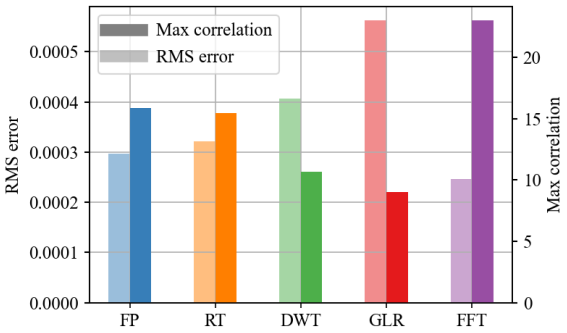

Figure 5: Performance evaluation on experimental data (mean of 3 recordings).

Through this study, we also compared two measurement paradigms, based either on event detection (FP, RT, DWT and GLR) or frequency detection (FFT). Each of the tested algorithms employs a distinct measurement approach : RT and FP are simple prominence-based detection methods implemented online and offline, respectively; GLRT utilizes a statistical test based on noise characteristics; DWT is a time-frequency method used as a filtering tool; FFT is a purely frequency-based approach. These approaches have specific advantages and drawbacks. RT and FP offer excellent temporal resolution, dynamics, and computational efficiency but exhibit poor robustness to noise and parameter changes. GLRT is more reliable than FP and RT provided that the excluded noise has Gaussian characteristics. DWT filtering presents a good trade-off between temporal resolution and performance; however, it still requires fine tuning relative to the signal. Finally, FFT consistently shows good performance : the sliding window implemented within the FFT approach reduces sensitivity to isolated noise events, as the analysis is performed over a window of samples rather than a single point. However this robustness comes at the expense of poor dynamics and temporal resolution, and therefore does not perform optimally during transient states. Another benefit of this algorithm is the weighted average of frequencies performed after the fft algorithm, which may be more relevant than threshold-based approaches. Indeed, SPs likely comprise multiple electrophysiological couplings at slightly different frequencies. To that extent, while event-based methods showed excellent performance, the frequency-based method may reveal supplementary information; indeed, islet behaviour result from multiple beta cell signals forming clusters of activation (Luchetti et al., 2023)(Jaffredo et al., 2018), with periodic behaviours well suited to frequency analysis. A frequency-based approach giving insight on the frequency spread of a signal rather than a single frequency measurement may thus be especially relevant to fully characterize islet behavior.

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